Standard Operating Procedure for the Determination of Cyanide

1.0 Scope and Applicability

This method is applicable to the determination of cyanide in drinking and surface waters, domestic and industrial wastes. The applicable range is 0.01 to 0.5 mg CN^-/L. The curve is linear. The method detection limit is 0.0012 mg CN^-/L. The precision as measured by the standard deviation is .00037 (determined from 8 replicates of a 0.008 ppm CN^- blank spike). See section 17 for more information.

2.0 Summary of Method

Fifty milliliters of a sample are placed into a glass distillation tube, which is part of a micro distillation setup. Fifty milliliters of a weak sodium hydroxide solution are added to a second tube which acts as a collection tube for the cyanide vapors. Cold water is turned on for the condensers. A vacuum line is connected and adjusted to the proper flow rate. Sulfamic acid, sulfuric acid and magnesium chloride are added to the distillation flask. The heater is turned on and the sample is allowed to reflux and distill over into the collection flask for 105 min. The apparatus is then allowed to cool for 15 min. The distillate is then poured into auto-sampler tubes and analyzed on the FIA against non-distilled standards. Distilled standards are run and must be within 10% of true value or a problem is indicated which must be corrected.

After the distillation step, which will remove most interferences, the cyanide ion is converted to cyanogen chloride (CNCl) by reaction with Chloramine-T at a pH less than 8. A red-blue color is formed upon the addition of the pyridine-barbituric acid reagent. The absorbance is read on the FIA colorimeter using the 570 nm interference filter.

Cyanides may be present in several forms and are classified according to the difficulty of the digestion step.

1. Free cyanide CN^-, HCN
2. Simple cyanide compounds
   a. readily soluble NaCN, KCN, $Ca(CN)_2$, $Hg(CN)_2$
   b. relatively insoluble $Zn(CN)_2$, CuCN, $Ni(CN)_2$, AgCN
3. Weak metal-cyanide $Zn(CN)_4^{2-}$, $Cd(CN)_3^-$, $Cd(CN)_4^{2-}$
4. Moderately strong cyanide $Cu(CN)_2^-$, $Cu(CN)_3^-$, $Ni(CN)_4^{2-}$, $Ag(CN)_2^-$
5. Strong metal-cyanide
   $Fe(CN)_6^{3-}$, $Fe(CN)_6^{4-}$, $Co(CN)_6^{4-}$, $Au(CN)_2^-$, $Hg(CN)_4^{2-}$

Although the EPA method measures total cyanide, it is not known if all of the above forms will be detected. *Standard Methods for the Examination of Water and Wastewater* suggests that strong metal-cyanides such as those listed in 5 above may not be detected.

3.0 Definitions

Reagent water - Water which has been run through a reverse osmosis system and then through de-ionization cartridges so that the final conductivity of the water is 17 meg ohm.

Calibration blank - A volume of reagent water fortified with the same matrix as the calibration standards, but without the analytes.

Instrument Performance Check Solution (IPC) - A solution containing the method analyte at the midrange of the standard curve. The purpose of this solution is to verify the calibration and monitor any potential drift of the instrument over the time of analysis.

Laboratory Fortified Blank (LFB) - An aliquot of reagent water or other blank matrices to which known quantities of the method analytes are added in the laboratory. The LFB is analyzed exactly like a sample, and its purpose is to determine whether the methodology is in control, and whether the laboratory is capable of making accurate and precise measurements.

Laboratory Reagent Blank (LRB) - Reagent water or other blank matrices that are treated exactly as a sample including exposure to all glassware, equipment, solvents, reagents, internal standards that are used with other samples. The LRB is used to determine if method analytes or other interferences are present in the laboratory environment, the reagents, or the apparatus.

Material Safety Data Sheet (MSDS) - Written information provided by vendors concerning a chemical's toxicity, health hazards, physical properties, fire, and reactivity data including storage, spill, and handling precautions.

Method Detection Limit (MDL) - The minimum concentration of an analyte that can be identified, measured and reported with 99% confidence that the analyte concentration is greater than zero. Defined according to section 16.4.

Quality Control Sample (QCS) - A solution containing a known concentration of the analyte usually from a source outside the laboratory and independent of the standards. The purpose of this solution is to verify that the standards are correct

Stock Standard Solution - A concentrated solution containing the method analyte

prepared in the laboratory using a purchased standard reference material, ideally traceable to NIST.

4.0 Interferences

Several interferences may be encountered with this method. Some of the known interferences are aldehydes, nitrate-nitrite, oxidizing agents such as chlorine, thiocyanate, thiosulfate, and sulfide. Some interferences are eliminated or reduced by the distillation.

Sulfides adversely affect the procedure by producing hydrogen sulfide during distillation. If a drop of the sample on lead acetate test paper indicates the presence of sulfide, treat 25 ml more of the stabilized sample (pH >12) than that required for the cyanide determination with powdered cadmium carbonate. Yellow cadmium sulfide precipitates if the sample contains sulfide. Repeat this operation until a drop of the treated sample solution does not darken the lead acetate test paper. Filter the solution through a dry filter paper into a dry beaker, and from the filtrate, measure the sample to be used for analysis. Avoid a large excess of cadmium and a long contact time in order to minimize a loss by complexation or occlusion of cyanide on the precipitated material.

High results may be obtained from samples that contain nitrate and/or nitrite. During the distillation nitrate and nitrite will form nitrous acid that will react with some organic compounds to from oximes. These oximes will decompose under test conditions to generate HCN. The interference of nitrate and nitrite is eliminated by pretreatment with sulfamic acid.

Oxidizing agents, such as chlorine, decompose most of the cyanides. Test a drop of the sample with potassium iodide-starch paper (KI- starch paper) at the time of collection; a blue color indicates the need for treatment. Add ascorbic acid, a few crystals at a time, until a drop of sample produces no color on the indicator paper; then add an additional 0.06 g of ascorbic acid for each liter of sample volume.

Aldehydes (such as formaldehyde) convert cyanide to cyanohydrin. Longer contact times between cyanide and the aldehyde and the higher ratio of aldehyde to cyanide both result in increasing losses of cyanide. If the presence of aldehyde is suspected, stabilize with NaOH at the time of collection and add 2 ml of 3.5% ethylene-diamine solution per 100 ml of sample collected.

Carbonate in high concentration may affect the distillation by causing excessive gassing when acid is added. The carbon dioxide released may significantly reduce the sodium hydroxide in the absorber. Use calcium hydroxide to preserve such samples. Add calcium hydroxide slowly, with stirring to pH 12 to 12.5. After the precipitate settles,

decant supernatant liquid for determining cyanide.

Some sulfur compounds may decompose during distillation, releasing S, H_2S or SO_2. Sulfur compounds may convert cyanide to thiocyanate and may also interfere with the analytical procedure for CN^-. To avoid this potential interference, add 5 mg $PbCO_3$ to the absorption solution before distillation. Filter the sample before proceeding with the colorimetric determination.

5.0 Safety

WARNING! Improper use of KCN can result in death. Free cyanide is a gas which when inhaled will attach itself to the red blood cells preventing the body from obtaining oxygen. This reaction is not reversible. Dissolve sodium hydroxide in water before adding potassium cyanide. Do NOT combine acid and potassium cyanide without the vacuum and hood running. Dispose of the 1000 ppm cyanide by running water in the sink first to wash out any acid then pour it down the drain with the water running.

The toxicity or carcinogenicity of each reagent used in this method has not been fully established. Each chemical should be regarded as a potential health hazard and exposure should be as low as reasonably achievable.

This method does not address all safety issues associated with its use. The laboratory is responsible for maintaining a safe work environment and a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material safety data sheets (MSDSs) should be available to all personnel involved in these analyses.

6.0 Equipment and Supplies

6.1 Lachat QuikChem 8000 Flow Injection Analyzer

6.1.1 96 place random access autosampler

6.1.2 Pneumatic pump

6.1.3 Colorimeter with:
10 mm flow cell
570 nm interference filter

6.1.4 Heater set for 60 deg C

6.1.5 Cyanide reaction module

6.1.6 Sample loop of 120 cm of .022 inch dia. teflon tubing

6.2 Gateway 2000 P5-60 computer

6.3 NEC Multisync 3FGe Monitor

6.4 HP 3Si printer

6.5 Andrews midi-distillation glassware (set of 10)

6.6 Vacuum pump

6.7 Omnion Software (ver. 2.0 Jan 99)

6.8 Analytical balance capable of accurately weighing to the nearest 0.0001g

6.9 Glassware - Class A volumetric flasks and pipets as required

7.0 Reagents and Standards

7.1 FIA Reagents

7.1.1 Carrier, 0.25 M NaOH: Add 20 g NaOH to 1600 mL reagent water in a 2 L beaker. Dissolve, dilute to 2 L and mix. Degas with helium.

7.1.2 Phosphate Buffer, 0.71 M KH_2PO_4: Dissolve 194 g anhydrous potassium dihydrogen phosphate in 1600 ml reagent water in a 2 L beaker. Dilute to 2 L and mix. Degas with helium.

7.1.3 Chloramine T: Dissolve 2.0 g Chloramine-T in about 250 ml reagent water in a 500 ml beaker. Dilute to 500 ml with reagent water and mix. Prepare fresh weekly.

7.1.4 Pyridine-Barbituric acid. In a fume hood, place 15.0 g barbituric acid in a 1 L beaker and add 100 ml water. Add 75 ml pyridine (C_5H_5N) with stirring. Add 15 ml concentrated HCl and stir until mostly dissolved. Dilute to volume. Barbituric acid will not go into solution completely until final volume is approached.

7.2 Distillation Reagents

7.2.1 Sulfuric Acid, 18 N: Slowly add 500 mL concentrated sulfuric acid to 400 mL reagent water in a large beaker surrounded with ice. When cool adjust the volume to 1 L with reagent water.

7.2.2 Magnesium Chloride solution: Dissolve 255 g of $MgCl_2.6H_2O$ in about 250 mL reagent water in a 600 ml beaker. Dilute to 500 ml with reagent water and mix.

7.2.3 Sodium Hydroxide solution, 1.25 N: Dissolve 50 g sodium hydroxide in reagent water in a 1 L beaker. Dilute to 1 L and mix. Use this solution for preparation of undistilled standards.

7.2.4 Sodium Hydroxide solution 0.25 N. Dissolve 20 g Sodium Hydroxide in a 2 L beaker. Dilute to 2 L with reagent water and mix. Use this solution for the receiving flasks.

7.2.5 Sulfamic Acid (NH_2SO_3H), crystals

7.3 Standards

7.3.1 Stock Cyanide Standard, 1000 mg CN/L (This is CN not nitrogen): Dissolve 2.0 g sodium hydroxide in about 500 mL reagent water in a 1 L volumetric flask. Add 2.5029 g potassium cyanide (KCN). CAUTION: KCN IS HIGHLY TOXIC. AVOID INHALATION OF DUST OR CONTACT WITH THE SOLID OR SOLUTIONS. Dilute to the mark with reagent water and invert three times. Prepare fresh weekly or when suspect.

7.3.2 Daily Stock Standard, 10 mg CN/L. Pipet 10 ml of the 1000 mg CN/L stock standard (7.3.1) into a 1 L volumetric flask. Add reagent water to the volume mark and mix..

7.3.3 Working Standards, non distilled. Add 20 ml of 1.25 N sodium hydroxide solution (7.2.3) to a series of 100 ml volumetric flasks and add the appropriate amount of the 10 mg/L Daily Stock Standard cyanide (7.3.2) from the table below. Bring to volume and mix.

ml stock STD	ppm of STD
5	0.5

2	0.2
1	0.1
0.5	0.05
0.1	0.01
0	0.00

8.0 Sample Collection, Preservation and Storage

Because most cyanides are very reactive and unstable, sample collection, preparation and preservation will be very important if meaningful results are to be expected. There are several concerns for sample integrity at field collection time. Some of these are oxidizing agents, oxidized products of sulfides, aldehydes, carbonates, temperature, and sunlight. If sample sources are known or suspected of containing any of the chemical interferences, special testing and treatment will need to be done upon collection. See section 4.0 on interferences.

Considerations: Approximately 1 L (500 mL minimum) of sample should be collected. Basic preservation consists of raising the pH of the sample to $\geq$12 with sodium hydroxide. If the samples contain chlorine or sulfide see Section 4.0. Samples should be analyzed as soon as possible after collection. If storage is required, preserved samples may be maintained at 4 deg C and stored in the dark for up to 14 days.

9.0 Quality Control

9.1 Initial Demonstration of performance

The Initial linear calibration range needs to be established over the concentration range used for analysis. At least three standards and a blank must be used. In the event of a non-linear response, additional standards will need to be run in order to accurately establish the curve. A quality control sample (QCS) needs to be run after the initial calibration and must run within +-10% of true value. Subsequent to the initial QCS, external QC samples will be run at least twice a year. If the QCS does not run within 10% of true value then the source of the problem must be identified and corrected before analyzing samples. The method detection level needs to be established using at least seven replicates of an analyte spike in reagent water and carried through the complete analysis. The MDL is calculated as:

$$MDL = (t) * (s)$$

Where t is the t value from the Student's t Table using the 99% confidence level

and n-1 degrees of freedom. The standard deviation, s is also calculated with n-1 degrees of freedom.

9.2 Assessing Laboratory Performance

Each batch of samples requires a laboratory reagent blank (LRB). Values which exceed the MDL indicate contamination and steps must be taken to correct this. At least one LFB must be analyzed with each batch of samples. The LFB must run within $\pm$ 10% to be in control. If more than 20 LFB's are tabulated in the data base a control chart using limits of $\pm$ 3 standard deviations may be used. These control limits must be equal to or better than $\pm$ 10%. A mid range calibration standard must be run immediately following the daily calibration and after every tenth sample. The acceptance limits are $\pm$ 10%. If the result for the standard is not within the acceptance limits, all samples following the last unacceptable calibration standard must be rerun.

9.3 Assessing analyte recovery and data quality

One sample in each group of ten or less must be spiked with the analyte at the same concentration as used in the LFB. 1.000 ml of the 10 mg CN/L Daily Stock Standard should be added to the flask. This will provide a spike of 0.2 mg CN/L. The percent recovery will be calculated as:

$R = [(C_s - C)/s]*100$

Where R = percent recovery
C_s = fortified sample concentration
C = sample background concentration
s = concentration of analyte added to sample

Results for spiked samples which fall outside the control limits when other control factors such as: the calibration curve, quality control samples, duplicates, LFBs, and the mid range check standard are running correctly indicate a sample matrix or solution problem.

One sample in each group of ten or less will be duplicated. If available Reference Materials should also be run to verify performance.

10.0 Calibration and Standardization

Prepare six standards, including a calibration blank, as outlined in section 7.3.3. Calibration is done by the automated colorimetric procedure in section 11.3. A calibration curve is fitted to the calibration solutions' concentration/response data using computer based regression curve fitting techniques. The curve should be linear, but tends to run second order if the reagents are not fresh (see section 17), and should run with a correlation coefficient of 0.999 or better. Failure at this point may be corrected by dropping one or two standards if they are serious outliers or rerunning the standards. If this does not correct the problem other solutions should be investigated including making up new standards. A more likely cause would be flow problems caused by obstruction in reagent lines or worn pump tubes. After the calibration has been established, it must be verified by the analysis of a midrange check standard / instrument performance check solution (IPC). If measurements exceed $\pm$10% of the established IPC value, the analysis should be terminated and the instrument re-calibrated. The new calibration must be verified before continuing analysis. A least two standards, a high and low range, are distilled and run against the non-distilled standards. They must agree with in $\pm$ 10%, otherwise the cause of the problem must be identified and corrected before proceeding. A least one quality control sample is also run and must be within the established control window.

The method is very sensitive to the matching of the sodium hydroxide concentration in the external standards with that in the receiving flask. Failure to match the sodium hydroxide concentration will result in checks, distilled standards and samples being outside the window of statistical acceptance.

11.0 Procedure

11.1 Distillation Procedure using Andrews glassware

11.1.1 Place the Andrews (10 place) midi-distillation unit under the hood.

11.1.2 Add 4-5 teflon boiling chips to the digestion flask.

11.1.3 Connect the cold water line and adjust to the proper flow rate (6 gal/hr). Remove any air bubbles in the cold finger by removing it and tilting it upright.

11.1.4 Place 50 ml of 0.25N NaOH into the receiving flask. Check the volume line on the receiving flask.

11.1.5 Place 50 ml of sample into the digestion flask.

11.1.6 Connect the main vacuum line to the pump, turn on the vacuum and make all connections to receiving and digestion flasks. Adjust the flow rate for each flask so that about 1-3 bubbles per second occur in the digestion flasks.

11.1.7 Turn on the hood fan.

11.1.8 Add 0.2g sulfamic acid to each digestion flask and rinse down. Allow to bubble for 3 minutes.

11.1.9 Slowly add 5 ml of 18 N H_2SO_4 to each digestion flask. Rinse down. Allow to bubble for 3 minutes.

11.1.10 Add 2 ml of $MgCl_2$ solution to each flask. Rinse down.

11.1.11 Turn the timer to 105 minutes and turn the heater on. At the end of the 105 minute period the unit will automatically cut the power. Wait 15 minutes before disconnecting the vacuum to the tubes and turning off the vacuum. The samples are now ready for the FIA.

11.2 Distillation of Standards. Add 45 ml of reagent water to each digestion flask and the following amount of 10 mg CN/L Daily stock standard from the table below for the standard you wish to distill.

ml 10 mg/L CN	Standard ppm
2.5	0.5
1.0	0.2
0.5	0.1
0.25	0.05
0.05	0.01

11.3 Automated Colorimetric Procedure

11.3.1 Install the cyanide manifold, (see section 17 for manifold diagram) run the feed lines through the pump cassette, and snap them into place. Turn on the pump. Place all feed lines into reagent water. Check for leaks.

11.3.2 Check that the temperature of the heater is at 60 deg C.

11.3.3 Instrument set up/method parameters

Chemistry	Direct
Injection to peak start	20
Peak base width	34.7
% width tolerance	15
Threshold	1200
Method cycle period	60
Probe in sample	28
Load period	16

11.3.4 Place standards, samples, and quality control checks into the auto sampler using the work list. (These are standards prepared in 7.3.3).

11.3.5 Degas all reagents except standards and the pyridine-barbituric acid solution with helium vigorously for one minute. Place all feed lines into proper reagent reservoirs. Pump reagents.

11.3.6 Open the proper method (cyanide.met) and Tray Table (cyanide.tra) on the FIA computer.

11.3.7 Transfer the worklist to the FIA computer, by exporting the worklist from a LIMS connected computer to a 3 ½ disk.

11.3.8 Import the work list from the 3 ½ inch disk to the FIA computer using the Start menu and selecting: “receive lognumbers”. At the prompt provide a file name such as the date: 0010180A. At the second prompt indicate that your first sample will start in position 7 following the standardization.

11.3.9 Check the tray table (work list) on the FIA computer for completeness of the transfer, dilutions that were made and delete any unwanted items left over from the previous tray. Save the tray table: “File, Save Tray”.

11.3.10 Start the analysis by clicking the “Run Tray” button on the main tool bar. On the next window click catalog on the file name line. On the next window type in your file name you used in 11.3.8. Click OK.

11.3.11 Check the baseline for stability. If the baseline is not flat, pause the run and restart the run when it is stable.

11.3.12 At the end of the run, rinse all the feed lines in reagent water for five minutes, then pump dry.

11.3.13 Print the calibration curve. From the tool bar click the graphic of the Calibration curve ”Review.” Select: “Print”, “Current Analyte.”

11.3.14 Evaluate the results of the quality control checks to determine if they are acceptable. If they are not acceptable, the source of the problem must be found and corrected and recalibration and reanalysis will need to be done before proceeding.

11.3.14 Print the custom report. From the tool bar click “Custom,” “Print,” “OK.”

11.3.15 Export the data file. Select: “File,” “Export Data”. Select channel 1 to 1 for export. Click ok.

11.3.16 Transfer the exported file back to a 3 ½ inch disk. From the Start Menu, select “Send Data”. Select transfer data to floppy. Select 1 -” import single channel data”. Type in the data file name you used in 11.3.8.

11.3.17 Import the FIA data to the LIMS.

11.3.18 Turn off the pump and all modules. Release the pump tube cassettes.

12.0 Data Analysis and Calculations

12.1 Calculations for the automated procedure are performed by the FIA computer using a least squares regression and the omnion ver 2.0 software. The calibration curve should be linear. Report sample results that fall between the high and low standard and only if the results for the related quality control checks are acceptable. Report results to three decimal places as mg CN/ L.

12.2 A correction needs to be made if an aliquot of the original sample or check solution was used for distillation.

Cyanide in sample = Cyanide from 12.1 x (50 mL)/(mL of aliquot used).

13.0 Method Performance

The method detection level was calculated using 8 replications of a 0.008 mg CN^- /L matrix spike. The MDL was 0.0012 mg CN/L with a precision of 0.00037 mg CN/L.

The bias at the spiked level was +1.9%.

14.0 Pollution Prevention

The following reagents have a potential for pollution: potassium cyanide, Pyridine, Barbituric acid, lead and cadmium carbonate (if used). To minimize pollution prepare only the minimum amount of reagents needed for use at that time.

15.0 Waste Management

It is the laboratory's responsibility to comply with all federal, state, and local regulations governing waste management, particularly the hazardous waste identification rules and land disposal restrictions, and to protect the air, water, and land by minimizing and controlling all releases from fume hoods and bench operations. Compliance with all sewage discharge permits and regulations is also required.

This is a reduced volume method and as such has reduced the volume of waste by a factor of 10. The primary waste is a 1000 mg/L cyanide standard which is disposed of down the drain with running water as are other chemicals listed in section 14.

For further information on waste management consult The Waste Management Manual for Laboratory Personnel and Less is Better: Laboratory Chemical Management for Waste Reduction, both available from the American Chemical Society's Department of Government Relations and Science Policy, 1155 16th Street NW., Washington DC, 20036.

16.0 References

16.1 Lachat QuikChem Method No. 10-204-00-1-A (Micro-Dist Method, May 1993).

16.2 *Standard Methods for the Examination of Water and Wastewater* 18th ed. 1992: 4500-CN^- A, B, C, and E.

16.3 EPA Method 335.4, Determination of Total Cyanide by Semi-Automated Colorimetry, EPA/600/R-93/100, August 1993, USEPA Office of Research and Development, Washington, D.C.

16.4 40 CFR Part 136 Appendix B- Definition and Procedure for the Determination of the Method Detection Limit.

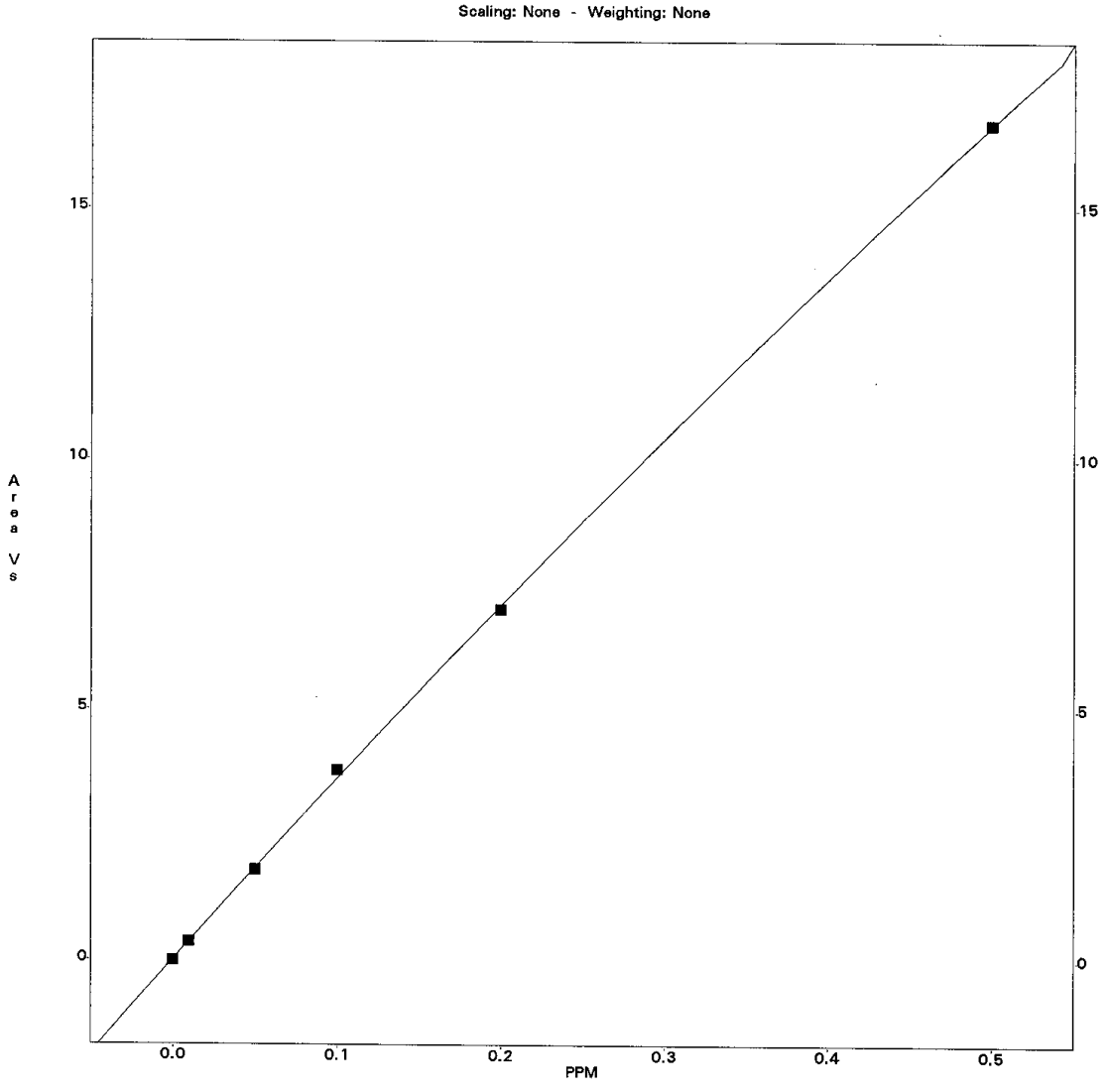
17.0 Tables, Diagrams, flowcharts, and Validation Data

Raw data to be saved includes the print out of the mg/L for the samples along with the printing of the regression analysis of the standards. After the data has been entered into the LIMS, the distribution sheet will also be kept. All records will be kept in the Cyanide book in order of date of analysis.

Cyanide Calibration Curve

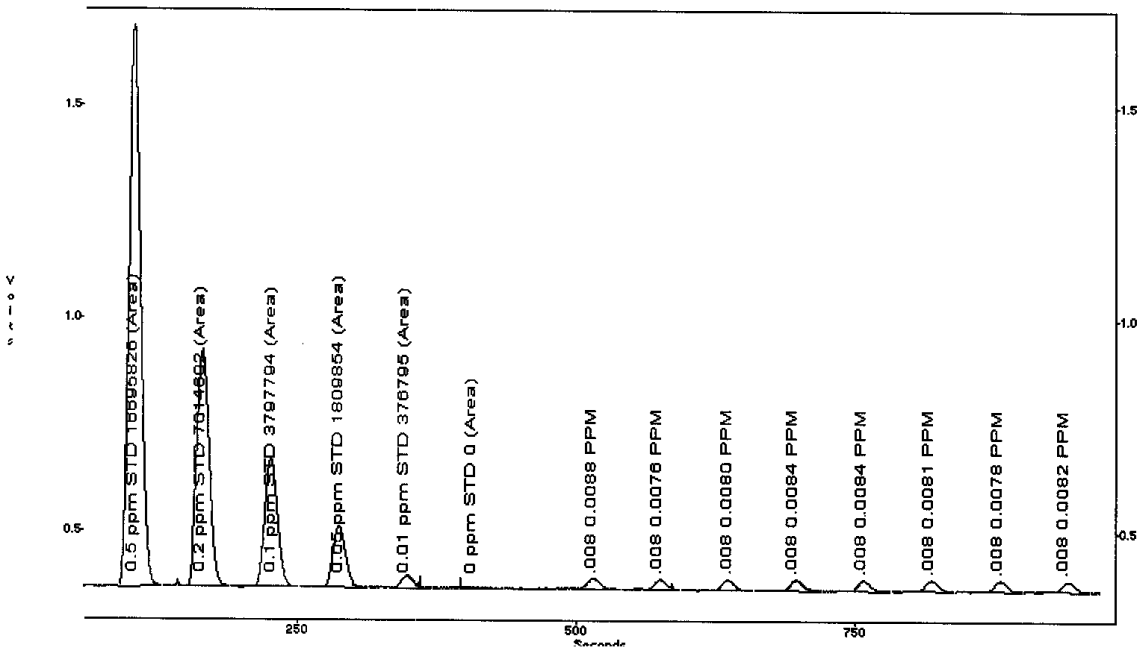
Lvl	Area	PPM	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Replic STD	Replic % RSD	Residual 2nd Poly
1	16695826	0.50	16695826					0.0	0.0	-0.1
2	7014602	0.20	7014602					0.0	0.0	1.3
3	3797794	0.10	3797794					0.0	0.0	-4.4
4	1809854	0.05	1809854					0.0	0.0	2.4
5	376795	0.01	376795					0.0	0.0	3.1
6	0	0.00	0					0.0	0.0	

2nd Order Poly
Conc = 1.849e-016 Area² + 2.690e-008 Area - 4.775e-004
r = 0.9999



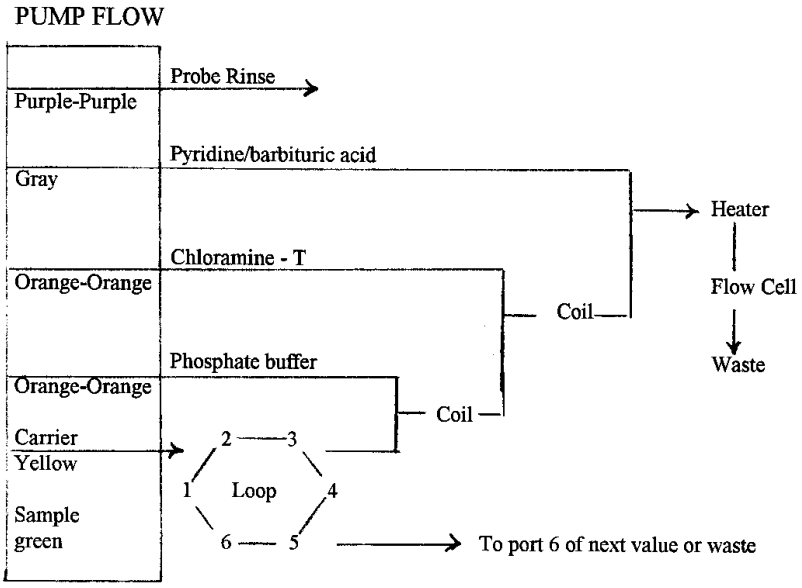
Printed: Monday, October 09, 2000 - 11:56 AM

MDL for Cyanide



n = 8
mean = 0.00815 mg CN/L
σ = 0.000117 mg CN/L
bias = +1.88%
MDL = (0.000117)(2.998) = 0.00117 mg CN/L

Cyanide Manifold Diagram



Notes

Sample loop = 120 cm of 0.032 inch inside dia PTFE tubing
Interference filter = 570 nm
Coils are 3/8 inch dia by 2 inch with 70 cm of 0.032 inch inside dia PTFE tubing
Pump speed = 30
Heater set at 60 deg C with 650 cm of 0.032 inch ID tubing.